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A THESIS

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL
OF CORNELL UNIVERSITY FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

BY
GEORGE FREDERICK POTTER

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PUBLISHED IN COLLABORATION WITH T. G. PHILLIPS
AS NEW HAMPSHIRE AGRICULTURAL EXPERIMENT STATION
TECHNICAL BULLETIN 42, JUNE, 1930

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SUMMARY

1. The composition in July and in August, 1925, of the new growth of non-bearing fruit spurs was determined in 26 plots of mature Baldwin trees under diverse cultural conditions. The proportion of spurs, similar to those analysed, which formed fruit buds was determined by actual counts made the following spring. The relation of composition to fruit bud formation was analysed by means of graphs and direct, partial and multiple correlation coefficients.

2. The spur constituent which proved to be most consistently associated with blossom formation is insoluble nitrogen. Conditions which lead to accumulation of this substance in large measure determined fruit bud formation.

3. In the aggregate conditions other than those favoring the accumulation of insoluble nitrogen have considerable bearing on fruit bud formation, but the samples were not numerous enough to permit very accurate measurement of relations other than that of nitrogen. There are indications that the accumulation of soluble carbohydrates, such as reducing sugars, depresses fruit bud formation. Data on a larger number of samples might be expected to bring out these minor relationships more clearly.

4. A tentative composite relation of fruit bud formation to seven spur constituents is given, by means of which fairly accurate estimates of expected performance can be made from the data on composition.

5. Carbohydrate-nitrogen ratios showed as close relations to fruit bud formation in most cases as nitrogen itself, but not significantly closer.

6. The accumulation of carbohydrates such as starch prior to July or during the period between samples was not an indication of conditions favorable to blossom differentiation.

7. Since in defoliation and shading low carbohydrate content is associated with low bloom, and in ringing high carbohydrate without increased nitrogen is associated with a high bloom, there is apparently a discrepancy between the results of such practices and the findings reported in this paper. This may be due to: (a) differences in absolute amount of nitrogen per spur or in the proportion of soluble to insoluble nitrogen not indicated by data on composition now available; (b) the increase or decrease of certain constituents to such an extent that their linear relation to fruit bud formation as determined from mature trees under field conditions no longer hold.

Composition and Fruit Bud Formation in Non-bearing Spurs of the Baldwin Apple*

In his monograph on fruit bud formation Summers (1924) aptly refers to blossoming in fruit plants as a response to a ''complex of external factors''. The effect of the more evident factors of this complex on blossoming has been studied for many years, but the responses are not yet well understood. More recently interest has centered on the immediate biochemical environment of the differentiating tissue, since it is believed that the more distant and general factors of the complex exert their influence through the changes they cause in the chemical composition of the spur. Summers calls this method an attempt to establish the ''biochemical foundation'' of the response. Some progress has been made, but a complete understanding of the biochemical factors involved is still very distant. The studies reported in this paper represent an attempt at further advance in this direction.

MATERIALS USED AND METHOD OF SAMPLING

Type of Spur. Bearing spurs of most varieties form few or no blossom buds. Non-bearing spurs are much more likely to form them. A number of investigators, including Hooker (1920, 1922), Kraybill et al (1925), Harley (1925), and Lagasse (1926), have made chemical analyses of bearing and non-bearing spurs. Usually the bearing spurs have been found to contain less carbohydrates and more nitrogen than the non-bearing spurs at approximately the same stage as regards fruit bud formation. Their failure to produce bloom has been explained on the basis of their low carbohydrate-nitrogen ratio, which would place them between conditions two and one as described by Kraus and Kraybill (1918). This condition would inhibit blossom production and even reduce growth. The non-bearing spurs are more nearly in condition three, in which because of better balance in supplies of nitrogen and carbohydrates, fruitfulness prevails.

Kraybill et al. (1925) found that the above relations held for the spurs from each of two plots of Baldwin trees. Both types of spurs from the sod plot, however, contained more carbohydrates and less nitrogen than the

*All the members of the staffs of the departments of Horticulture and of Agricultural and Biological Chemistry helped with the sampling. S. W. Wentworth assisted with the extractions, G. P. Percival and P. T. Blood made a large number of the chemical determinations, and G. M. Hartshorn furnished valuable aid in the calculations. Dr. A. J. Heinicke has read the manuscript and made helpful suggestions.

corresponding types from a cultivated plot heavily fertilized with nitrogen. As a result, the carbohydrate-nitrogen ratios of the bearing spurs from the sod plot, and the non-bearing spurs from the cultivated plot, were similar. None of the former spurs blossomed, but 40 per cent of the latter produced flowers.

Lagasse (1926) obtained similar results in a study of the spurs from plots of Jonathan trees receiving different amounts of nitrogenous fertilizer. The proportion of bearing spurs producing flower primordia was less than half that of the non-bearing spurs of similar composition.[1]

It would seem that the comparative composition of bearing and non-bearing spurs is not a good index of their relative fruitfulness. This may be due to the nutritional dominance of the growing fruit on the spur system which Murneek (1928) has emphasized. The bearing spur includes a rather large mass of specialized tissue, the "cluster base" or "dard." It is quite possible that this differs in composition from that portion of the spur directly surrounding the developing bud, and that the composition of the whole spur does not represent the nutritive conditions surrounding the bud. Apparently these portions of the spur have not been separated in any of the studies so far reported. To avoid these complications it seemed best in this case to limit the investigation to non-bearing spurs.

Portion of Spur. It would be very desirable to determine the composition of that minute portion of the spur directly surrounding the tissue which may form a blossom bud. This cannot be done with the methods available. The best that can be done is to approach this as nearly as pos-

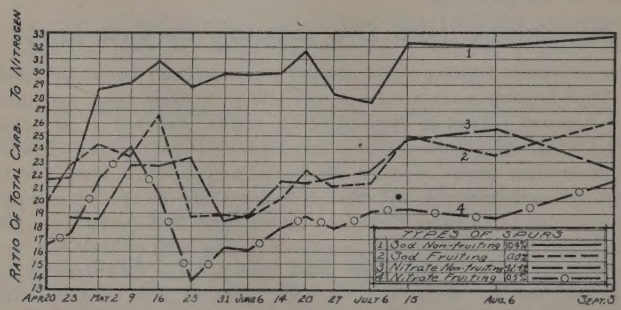

Figure 1. Variations in ratios of total carbohydrates to total nitrogen in the four spur types at different dates during the season. Bearing spurs (line 2) form no fruit buds, although almost identical in composition with non-bearing spurs (line 3) forming many. Figure 18 from N. H. Agr. Exp. Sta. Tech. Bull. 29, 1925.

sible and to make the various samples taken as nearly uniform in their relation to this active region as possible. Harley (1925) found that the composition of the older portions of wood is comparatively variable. This

[1]Private communication, June 13, 1927, in files of G. F. P.

might seem to reflect on the validity of the results of Kraybill et al. (1925) who included two or three years old growth with that of the current season in their samples. However, analyses of successive random samples of the same types of spurs agreed rather well and the carbohydrate-nitrogen relations of the bearing spurs from the sod plot and the non-bearing spurs from the nitrogen plot were consistently similar. At all times after May 16 they were intermediate between the non-bearing spurs of the sod plot and the bearing spurs of the nitrogen plot. (See Fig. 1)

To avoid the inclusion of the older wood and of cluster bases which would be present on the older growth of many of the non-bearing spurs, it was decided to use only the current season's wood in this study.

Harley also found that spurs of uniform length were less variable chemically than those of random sizes. It would seem desirable, therefore, to limit the samples to spurs of uniform length. This was impracticable because it was necessary to take about 1000 spurs from each group of trees, 250 for each individual sample, and leave enough of the same type to make possible a satisfactory estimate of performance the following spring. It should be noted that Harley's samples consisted of 30 spurs. On the basis of random sampling, different lots of 30 spurs each would be about three times as variable as lots of 250 gathered from the same source. No definite limit was placed on the length of the spurs sampled, but they did not vary in length as widely as did Harley's "non-uniform" samples.

Trees Sampled. Differences in the per cent of spurs forming fruit buds exist between almost any two plots of trees, and at the same time a number of differences in fruit spur composition. It does not necessarily follow that all the chemical differences observed are concerned in, or even usually associated with, a difference in reproductive response.

It is possible, also, that different chemical situations may bring about similar responses in blossom bud formation. It is well known, for instance, that many factors other than pollination influence set of fruit. Just as Heinicke (1917) demonstrated by means of seed counts that vigorous spurs poorly pollinated may set about as much fruit as weak spurs well pollinated, so in fruit bud formation there may be a certain degree of compensation between favorable and unfavorable chemical influences. Reliable results can hardly be secured, then, from a study of a small number of samples. Relations due to mere coincidence cannot be distinguished from those that are really significant. Only such differences in composition as are found consistently associated with scarce or abundant fruit bud formation in a large number of observations can be considered significant.

In this study it was decided, therefore, to take as many samples as the limits of time and reasonable uniformity of material would permit. Thus the twenty-six plots of mature Baldwin trees, 30 to 45 years old, and which were available near the Experiment Station, were sampled in the season of 1925. In Table 1 these plots are designated, for the purpose of this discussion, by the percentage of spurs which were found to have formed fruit buds. Random counts were made, in May 1926, of the performance of spurs which were similar in type and in distribution on the tree to those

TABLE 1.—*Cultural Treatment of Baldwin Plots*

Per cent of Non-bearing Spurs Forming Fruit Buds†	Soil Management	Pounds of Fertilizer Applied Annually per tree						Division Row Between Plots
		Nitrate of Soda	Muriate Potash	Sul-phate Potash	Super-phos-phate	Basic Slag	Stable Manure	
2.7±3.6	Cult. 1925 & alternate yrs.							
8.3±3.6	Cult., no cover crop							
13.7±4.1	Sod mulch	5						
14.2±3.6	Cultivated	2		10	8½			
15.7±3.8	Cultivated							
16.4±3.6	Cultivated	2		4	8½			
18.4±3.6	Cultivated	2		4	17			
18.5±3.6	Sod mulch	8						
19.1±3.6	Sod mulch							
20.8±3.6	Sod mulch	12*						
22.3±3.6	Cultivated							34.5—14.2
25.2±3.6	Cultivated	2		4		7		
28.7±4.8	Sod stone wall	10						
29.4±4.4	Cultivated	5	3		7			
33.3±4.4	Sod mulch							59.2—13.7
34.5±3.6	Cultivated	6		4	8½			
34.6±4.8	Sod mulch							38.5—13.7
35.8±4.1	Cultivated							44.5—29.4
38.5±4.4	Sod mulch						400	
44.5±4.1	Cultivated	5						
50.1±4.1	Cultivated							29.4—38.5
53.6±4.1	Cultivated						400	
55.0±3.8	Cult. alternate yrs. sod 1925							
57.6±4.8	Sod stone wall**	8						
57.8±4.1	Sod stone wall**	8						
59.2±4.1	Sod mulch	5	3		7			

*8 lbs. early May, 4 lbs. late July.
**Garden adjacent.
†Buds initiated in 1925, blossoms opening and counts made May 1926.

removed for chemical analysis in 1925. Altogether nearly 100,000 spurs were counted in determining these percentages.

It is evident that the trees were under the most diverse cultural conditions, ranging from sod culture with no fertilizer to thorough cultivation and heavy applications of fertilizer. A cover crop of buckwheat was used on all plots which were cultivated with the exception of Plot 8.3 which has been clean-cultivated for a number of years. The plots are those of various permanent experiments. In five cases trees were used from a division row between two fertilizer treatments. In many cases not all of the trees receiving a given treatment were used, but rather five to nine of those which were bearing the least fruit and which appeared most similar in other respects. It was considered of first importance to obtain uniform material in each sample, and in general smaller variations in fruit bud formation will be found in a smaller number of trees than in a greater number. Trees in heavy bearing, on which only a few non-bearing spurs would be found, were avoided.

Time of Sampling. The work of Kraybill, et al. (1925) showed that chemical differences which may be associated with fruit bud formation

appeared about the end of June or the first of July and became somewhat more evident in August. In order to be sure to secure spurs when marked differences would be found it was thought best to sample twice, early in July and early in August. With the aid of as large a crew as could be used conveniently, each sampling of the 26 plots required seven or eight days. The order in which the plots were sampled was the same in both cases so that the interval between the first and second sample was the same for each plot. The first collection of spurs will be referred to as the July sample, the second as the August sample. The actual sampling occurred between the first and tenth of each month.

A survey of the literature now available shows that the July sample is probably the more important. In this country Goff (1899, 1900, 1901), Drinkard (1910), Bradford (1915), Kirby (1918), and Tufts and Morrow (1925) have reported on the time when fruit bud differentiation in the apple occurs. None of these studies applies to Baldwin trees in this latitude. In general, their results would indicate that differentiation begins rather early, in late June or early July, even in the latitude of Southern Wisconsin. However, there seems to have been a tendency to emphasize the first bud found rather than the season at which the majority make their appearance.

Recently Rasmussen (1929) has published the results of an extensive study of the time of differentiation of fruit buds in the locality of Durham, N. H. By examining about 100 buds each week he was able to determine with some accuracy not only the time of earliest differentiation but also the period during which the process continued. In 1928 the first definite indications of blossoms in the Baldwin were in samples picked August 7, and the proportion of flower buds increased for about three weeks. In 1929 the period of differentiation was of about the same length, but began and ended about a week earlier than in 1928.

It seems evident that the conditions favoring fruit bud formation must have been effective at some time previous to the first evident differentiation. Harvey and Murneek (1921) found that the defoliation of spurs June 16 and 17 resulted in markedly decreased formation of blossom buds, and that chemical differences between defoliated spurs and checks were evident in samples taken July 5. Kraybill (1923) found that ringing eight-year-old McIntosh trees on June 1 resulted in increased fruit bud formation, and in chemical differences which were evident in samples of spurs taken July 8. The non-bearing trees which Shaw (1923) ringed late in May showed markedly increased fruit bud formation. Those ringed in June showed less response, and those treated in July showed practically no results. Shading experiments conducted by Paddock and Charles (1928) seemed to indicate that inception of blossom buds cannot be influenced by this treatment later than two weeks after bloom.

With the plum, Roberts (1923) found that defoliation depressed fruit bud formation decreasingly from June 16 to July 10, and that defoliation on July 24 was not effective. The buds were visible August 8. In all these cases at least a short time would be required for the treatment to

affect the chemical composition of the structure concerned. That conditions favoring reproductive response may have their effect long before evident differentiation occurs is shown by the work of Thompson (1929) on premature seeding in celery. Plants that had been subjected to the 30-day low temperature treatment showed evidences of seed stalk development about three months later. Chemical differences were marked immediately after the treatment and were much less pronounced at the time when seed stalks were evident.

In the spurs used in this study it seems evident that if chemical differences have an influence in determining fruit bud formation they must be present early in July. If chemical differences in the August samples are found to be associated with fruit bud formation the relationship must be considered as parallel rather than causal.

To avoid diurnal changes in starch and other constituents, the samples should all be picked at as nearly as possible the same hour of the day, but to complete the work before large seasonal changes could occur, it was necessary to secure several samples a day. The spurs were collected usually between 8 and 11 o'clock each day, though in some instances the day's quota was not obtained until noon. An equal number of spurs was taken from each tree. These were picked either by hand or with pruning shears from representative positions scattered throughout the entire bearing surface. As the spurs were dropped by the picker they were gathered and the leaves removed immediately. One-half of the spurs from each tree were placed in each of two stoppered flasks which had previously been washed, dried and weighed. Thus duplicate samples were taken on each date from each plot. The samples in the flasks were taken to the laboratory for preservation at the close of each day's picking.

METHODS OF CHEMICAL ANALYSIS

Preserving the Samples

As soon as a stoppered flask containing a sample was brought into the laboratory it was weighed. The spurs were emptied onto glazed paper and cut, with pruning shears, as rapidly as possible into sections from one to three mm. long. The sample was returned to the flask; 0.25 g. $CaCO_3$ and 150 cc. hot, redistilled 95% alcohol were added, and the flask was heated on the water bath to gentle boiling for 30 minutes. After cooling, the flask was stoppered with a cork protected by tin foil, and set aside until the extraction could be begun.

Extraction

The alcohol was filtered from the sample through a hardened paper that had been washed with 80% alcohol, into a 500 cc. calibrated flask. Care was taken to get as little of the solids as possible on the paper. The residue in the flask was just covered with 80% alcohol, brought to boiling on the water bath, allowed to cool to room temperature, and the alcohol was filtered through the same paper. Each sample was extracted seven times in this way. Preliminary tests had shown that only negligible amounts of

material were dissolved by further extractions. The filtrate and washings were brought to volume with 80% alcohol at 20°, mixed and transferred to a storage bottle which was stoppered with a cork covered with tin foil.

Treatment of the Residue

The residue in the flask was transferred to a weighed 150 cc. beaker, using 80% alcohol to transfer the last traces. The solids on the filter paper were transferred similarly to the same beaker. The alcohol was evaporated off on the water-bath and the residue was dried to constant weight in an electric oven at 102° to 103°. The dry residue was ground to pass an 80-mesh sieve, using a Nixtamal mill and finishing in an iron mortar. Moisture was determined in duplicate one gram portions and the remainder was placed in a vial which was stoppered with especial care to avoid changes in moisture until further portions were weighed for analysis.

Analysis of the Extract

Solids. Duplicate 15 cc. aliquots of the extract were measured into weighed 50 cc. beakers, and evaporated on the water bath to a thick syrup. The drying was completed in the vacuum oven at 70°.

Sugars. Reducing power was determined by Fehling's solution, using the modifications and conditions proposed by Quisumbing and Thomas (1921) except that the reduction was made in 250 cc. beakers. The cuprous oxide was determined by the Bertrand method, using an acid solution of ferric alum as the solvent, and approximately 0.05 N $KMnO_4$ for the titration. A daylight lamp was used for the titration which was carried to a distinct pink color. The blank found by titrating to the same color amounted to 0.5 cc. of the $KMnO_4$. No trouble was experienced with uncertain end points if the cuprous oxide had been washed thoroughly. The copper equivalents of small amounts of glucose and sucrose were determined, using Bureau of Standards samples. For 10 mg. or more of glucose and 15 mg. or more of sucrose these results agreed satisfactorily with those of Quisumbing and Thomas. The results obtained were used to construct tables for amounts of the sugars smaller than those mentioned.

Preliminary Treatment. Duplicate 100 cc. aliquots were used. The aliquot was transferred to a 250 cc. beaker and evaporated to a small volume on the water bath. Two or three successive small portions of water were added with intervening evaporation until the alcohol was removed completely. The material in the beaker was transferred to a 250 cc. calibrated flask. Water was added nearly to volume and then 1 cc. of standard neutral lead acetate solution. The sample was then diluted to volume, mixed thoroughly, and after standing at least 15 minutes, filtered through a dry paper into a dry flask. To this filtrate, 0.20 g. anhydrous sodium oxalate were added. After shaking thoroughly and allowing to settle, the mixture was filtered through a dry paper into a dry flask. The sugar determinations were made on aliquots of this filtrate, which was preserved with toluene.

Direct Reducing Sugars were determined in a 50 cc. aliquot of this filtrate.

Sucrose. To a 50 cc. aliquot of the above filtrate in a 250 cc. beaker, two drops of Methyl Red indicator were added and the reaction was adjusted to a pH of approximately 5.0. Seven drops of Wallerstein's dialized invertase preparation were added, with stirring. Reducing power was determined after the enzyme action had continued for at least one hour and fifteen minutes. The sucrose equivalent of the copper obtained by direct reduction was subtracted from the sucrose equivalent of the copper obtained after inversion. The difference was considered actual sucrose.

Phlorhizin was determined by a modification of the method of Harvey (1925). A 25 cc. aliquot of the deleaded filtrate, 25 cc. of water and 10 cc. of 12% HCl were transferred to a 100 cc. Erlenmeyer flask which was held in the water-bath at 80° for three hours. After cooling, the solution was neutralized to litmus with 50% NaOH. The final adjustment of reaction was made with 10% NaOH or 12% HCl as required. The neutral solution was transferred to a 100cc. calibrated flask, diluted to volume, and mixed.

Reducing power was determined in a 50 cc. aliquot. The glucose equivalent of the copper reduced was multiplied by 100 to convert it into terms of the whole sample. From this was subtracted the sum of the glucose (direct reducing) and sucrose in the whole sample, to give the glucose equivalent of the phlorhizin. This result was multiplied by 2.42 to obtain the value for anhydrous phlorhizin. Since the copper equivalent of glucose and of sucrose (expressed as sucrose) are almost identical the error introduced by this method of calculation is very small.

Nitrogen. Careful semi-quantitative tests on suitably cleared portions of several of the extracts showed that in no case was more than one part per million of nitrate nitrogen present. It was thought unnecessary, therefore, to modify the method to include nitrates. Total nitrogen was determined in a 50 cc. aliquot of the alcoholic extract.

The H_2SO_4 (20 cc.) and catalyst ($CuSO_4$ and $HgSO_4$) were added to the aliquot. The preliminary heating was done slowly and carefully as bumping and frothing were quite severe. As the amounts of nitrogen to be determined were very small, care was used in selecting reagents as free as possible from nitrogen. Distillations were made through Pyrex Davisson scrubber bulbs and adapters connected to block tin pipes, into 0.02N H_2SO_4. The excess acid was titrated to Methyl Red with 0.02N NaOH. Blank determinations gave nitrogen equivalent to 0.7 cc. of 0.02 N NaOH.

Analysis of the Insoluble Residue.

Carbohydrates. A 2 g. sample of the insoluble residue was transferred to a filter paper in a funnel and washed into a 250 cc. calibrated flask with water until the washings nearly filled the flask. The washings were brought to volume and mixed. They were practically free from direct reducing substances. A 50 cc. aliquot of the washings and 10 cc. 24% HCl were transferred to a 100 cc. Erlenmeyer flask and heated in the water bath at 80° for three and one-half hours. The solution was neutralized, transferred to a 100 cc. calibrated flask and diluted to volume. Reducing power was determined in a 50 cc. aliquot. The results were calculated as $C_6H_{10}O_5$ and called Water Soluble Acid Hydrolyzable Material.

Starch. The residue from the above treatment, while still moist, was washed into a 250 cc. wide mouth Erlenmeyer flask. The volume was made up to 190 cc. and the flask was heated in the boiling water bath for 30 minutes. After cooling, 10 cc. (equivalent to 0.02g.) of a solution of undiluted Taka Diastase* were added. The mixture was protected by toluene and incubated at 38° for 24 hours. The flask was then heated in the boiling water bath 15 minutes to destroy the enzyme. When cool, the mixture was filtered into a 500 cc. calibrated flask and washed nearly to volume. The filtrate and washings were brought to volume and a 50 cc. aliquot was heated with 10 cc. 24% HCl in a water bath at 80° for three and one-half hours. Preliminary tests with pure maltose had shown that this treatment gives the maximum yield, approximately 96% of the theoretical. After cooling and neutralizing, the solution was diluted to 100 cc. and reducing sugars were determined in a 50 cc. aliquot. The result was calculated as $C_6H_{10}O_5$ and called Starch.

Acid Hydrolyzable Polysaccharides

The insoluble residue from the starch determination was transferred to a 250 cc. wide mouth Erlenmeyer flask and water was added to a total volume of 100 cc. After adding 20 cc. 12% HCl the flask was heated under an air condenser for two and one-half hours in a boiling water bath. After cooling and neutralizing to the natural indicator the mixture was filtered into a 500 cc. calibrated flask and washed nearly to volume. The reaction of the mixture was adjusted more closely, using Methyl Red, and the volume was brought to 500 cc. Reducing sugars were determined in a 50 cc. aliquot. The result calculated as $C_6H_{10}O_5$ was called Acid Hydrolyzable Polysaccharides.

Nitrogen was determined by the method used for the analysis of feeding stuffs in duplicate one gram samples of the insoluble residue.

In all the calculations it was assumed that the 0.25 g. $CaCO_3$ remained entirely in the insoluble residue.

THE RESULTS OF CHEMICAL ANALYSIS

All the determinations except those of fresh weight and insoluble solids were made in duplicate. As duplicate samples were taken at each period, each figure in Tables 2 to 5, with the exceptions noted, is the average of four determinations. The results were calculated and studied on three bases: Weight per 100 Spurs, Per Cent Fresh Weight and Per Cent Dry Weight. To save space the results in per cent dry weight are not presented.

The duplicate determinations on the same sample checked very closely in every case. In some instances greater differences were found between duplicate samples, but in the main these were not large. An individual probable error could be calculated for each of the figures given, but Engledow and Yule (1928) have suggested that little confidence can be placed in the rather diverse probable errors which would be obtained for

*Commercial Taka Diastase is diluted to standard strength with lactose, and therefore gives a large blank in the starch determination. Parke, Davis & Company very kindly supplied an undiluted preparation which was more than five times as active as the standard, and gave no blank.

TABLE 2.—*Results of Chemical Analysis*
July Sample

Per Cent Fruit Buds Formed	Weight per Hundred Spurs												
	Fresh Weight	Soluble Solids	Insoluble Solids	Total Solids	Reducing Sugars	Sucrose	Phlorhizin	Water Soluble Acid Hydrolyzable	Starch	Acid Hydrolyzable	Soluble Nitrogen	Insoluble Nitrogen	Total Nitrogen
	Grams	Grams	Grams	Grams	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
2.7	10.126	1.34	2.982	4.32	89.2	30.4	529	35.35	95	480	2.71	30.8	33.5
8.3	10.666	1.31	3.168	4.48	94.1	24.0	569	62.84	147	514	2.80	32.5	35.3
13.7	13.662	1.67	4.086	5.76	105.4	39.4	708	47.66	179	620	6.50	43.2	49.7
14.2	10.568	1.29	3.066	4.35	81.0	28.0	508	45.49	157	496	3.46	30.8	34.3
15.7	12.258	1.52	3.062	5.12	105.6	35.4	615	82.30	182	584	3.42	36.2	39.6
16.4	12.338	1.44	3.614	5.06	92.6	31.4	589	74.60	218	600	3.34	35.9	39.2
18.4	11.948	1.39	3.497	4.88	92.3	34.6	542	78.08	203	565	3.24	34.0	37.2
18.5	11.124	1.41	3.441	4.84	86.2	20.2	572	72.54	186	444	4.06	33.6	37.7
19.1	10.446	1.28	3.110	4.40	80.8	32.5	559	48.10	110	491	2.48	31.8	34.3
20.8	10.952	1.40	3.454	4.86	86.1	24.6	585	95.00	178	524	4.44	33.7	38.1
22.3	10.352	1.22	2.974	4.20	76.2	25.6	494	69.72	156	426	3.04	30.1	33.1
25.2	11.726	1.31	3.270	4.58	86.8	22.4	538	76.47	183	523	2.90	33.9	36.8
28.7	10.578	1.38	3.080	4.46	72.8	34.2	564	57.66	133	470	5.43	35.5	40.9
29.4	12.464	1.51	3.554	5.06	100.4	32.0	619	52.10	177	518	10.16	41.6	51.8
33.3	11.234	1.37	3.258	4.63	76.6	32.2	566	45.32	151	506	3.68	32.8	36.5
34.5	11.003	1.29	3.117	4.40	86.8	24.1	492	58.35	170	464	4.05	34.2	38.2
34.6	13.026	1.61	3.984	5.60	104.4	32.0	665	57.46	191	656	5.16	38.0	43.2
35.8	14.406	1.72	4.256	5.98	120.6	34.6	686	65.96	197	677	7.68	44.9	52.6
38.5	13.071	1.63	3.992	5.62	97.7	35.8	640	52.08	199	662	5.54	38.8	44.3
44.5	14.006	1.68	4.188	5.87	130.2	28.0	680	85.15	213	736	7.05	45.6	52.6
50.1	13.520	1.65	4.034	5.68	115.6	24.2	661	65.77	240	604	5.33	42.6	47.9
53.6	14.649	1.68	4.311	5.98	119.5	32.2	678	56.92	239	694	6.56	44.8	51.4
55.0	13.229	1.61	3.803	5.41	116.0	36.2	644	52.20	156	618	4.05	41.8	45.8
57.6	15.942	1.95	4.936	7.46	123.2	47.6	823	60.80	277	795	7.46	52.2	59.7
57.8	10.403	1.28	3.177	4.46	75.2	22.6	517	74.17	185	496	6.36	33.6	40.0
59.2	11.994	1.45	3.443	4.90	78.0	27.7	598	57.98	132	516	4.18	37.7	41.9

TABLE 3.—*Results of Chemical Analysis*
August Sample

	Weight per Hundred Spurs												
Per Cent Fruit Buds Formed	Fresh Weight	Soluble Solids	Insoluble Solids	Total Solids	Reducing Sugars	Sucrose	Phlorhizin	Water Soluble Acid Hydrolyzable	Starch	Acid Hydrolyzable	Soluble Nitrogen	Insoluble Nitrogen	Total Nitrogen
	Grams	Grams	Grams	Grams	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
2.7	9.332	1.23	3.304	4.53	58.0	34.4	526	32.94	203	444	2.76	31.3	34.1
8.3	11.660	1.44	4.090	5.54	78.5	35.2	638	41.10	256	616	3.67	38.4	42.1
13.7	8.692	1.13	3.140	4.28	58.9	25.9	494	31.38	128	414	3.31	32.8	36.1
14.2	10.694	1.24	3.657	4.90	60.8	23.1	530	34.19	230	482	4.05	35.4	39.4
15.7	11.888	1.42	2.966	5.38	83.0	17.8	662	46.03	236	588	3.50	38 0	41.5
16.4	11.406	1.34	3.818	5.16	68.2	27.6	589	30.66	247	527	4.14	37 6	41.7
18.4	11.524	1.37	3.900	5.27	73.0	35.8	568	37.04	233	585	4.74	37 3	42.0
18.5	9.061	1.22	3.378	4.60	66.3	29.8	578	31.59	196	414	3.07	34.2	37.3
19.1	10.072	1.27	3.532	4.80	61.4	29.0	552	40.94	236	483	2.36	32.1	34.5
20.8	10.600	1.38	4.028	5.41	76.2	37.2	557	48.46	237	603	4.52	38.8	43 3
22.3	13.034	1.46	4.336	5.80	84.8	21.0	639	39.16	213	659	5.17	43.0	48 2
25.2	12.285	1.42	4.079	5.50	72.3	25.6	610	33.95	269	598	4.74	41.3	46 0
28.7	9.630	1.26	3.370	4.63	58.9	23.7	536	34.06	190	480	4.44	38.6	43.0
29.4	11.666	1.36	3.860	5.22	71.7	24.9	684	32.26	177	480	7.34	44.6	51 9
33.3	12.130	1.53	4.379	5.91	69.2	37.7	655	48.78	239	684	4.30	43 1	47 4
34.5	13.042	1.45	4.231	5.68	78.6	30.4	610	39.21	173	642	6.66	44.0	50 7
34.6	11.484	1.42	4.068	5.48	69.6	29.8	615	43.62	222	586	4.30	39.4	43.7
35.8	12.590	1.45	4.167	5.62	81.4	20.6	650	37.18	238	551	6.18	47 2	53 4
38.5	13.800	1.62	4.687	6.31	76.9	29.7	675	40.55	302	658	6.06	47 3	53 4
44.5	14.274	1.73	4.937	6.66	103.8	31.9	715	47.57	279	720	8.30	52.8	61.1
50.1	12.136	1.49	4.025	5.51	76.4	22.5	637	43.24	233	562	6.24	43.3	49 5
53.6	14.518	1.69	4.894	6.58	94.3	31.6	676	42.38	296	642	8.24	51.6	59.8
55.0	13.812	1.73	4.868	6.60	98.7	48.0	740	59.90	329	727	4.68	47.2	51.9
57.6	10.492	1.40	3.738	5.14	58.7	29.4	595	32.40	191	571	4.98	42.4	47.4
57.8	9.820	1.22	3.404	4.62	53.8	27.6	488	29.55	207	430	5.26	39 1	44.4
59 2	14.820	1.73	5.266	7.00	90.6	37.3	717	51.22	280	776	5.66	52.5	58 2

TABLE 4.—*Results of Chemical Analysis*
July Sample

Per Cent Fruit Buds Formed	Fresh Weight grams per 100 Spurs	Per Cent Fresh Weight											
		Soluble Solids	Insoluble Solids	Total Solids	Reducing Sugars	Sucrose	Phlorhizin	Water Soluble Acid Hydrolyzable	Starch	Acid Hydrolyzable	Soluble Nitrogen	Insoluble Nitrogen	Total Nitrogen
2.7	10.126	13.2	29.4	42.6	.878	.301	5.225	.349	0.94	4.74	.0267	.304	.331
8.3	10.666	12.4	29.7	42.1	.882	.224	5.335	.589	1.38	4.82	.0262	.305	.331
13.7	13.662	12.2	29.9	42.1	.771	.340	5.187	.348	1.32	4.54	.0476	.316	.364
14.2	10.568	12.2	29.0	41.2	.762	.266	4.811	.430	1.48	4.69	.0328	.292	.325
15.7	12.258	12.4	29.4	41.8	.862	.289	5.015	.672	1.48	4.76	.0280	.295	.323
16.4	12.338	11.6	29.3	40.9	.750	.254	4.774	.604	1.76	4.92	.0270	.292	.319
18.4	11.948	11.6	29.3	40.9	.773	.288	4.533	.654	1.70	4.72	.0272	.284	.311
18.5	11.124	12.6	30.9	43.5	.774	.182	5.146	.652	1.67	4.87	.0366	.302	.339
19.1	10.446	12.3	29.8	42.1	.770	.311	5.360	.460	1.06	4.70	.0238	.304	.328
20.8	10.952	12.8	31.5	44.3	.786	.224	5.337	.868	1.62	4.78	.0406	.307	.348
22.3	10.352	11.8	28.7	40.5	.736	.248	4.771	.674	1.50	4.10	.0298	.291	.321
25.2	11.726	12.2	27.9	40.1	.741	.190	4.592	.652	1.56	4.46	.0247	.289	.314
28.7	10.578	13.0	29.1	42.1	.688	.324	5.330	.545	1.25	4.45	.0514	.336	.387
29.4	12.464	12.1	28.5	40.6	.806	.256	4.924	.418	1.42	4.16	.0815	.334	.416
33.3	11.234	12.2	29.0	41.2	.682	.286	5.035	.402	1.34	4.51	.0326	.292	.325
34.5	11.003	11.6	28.3	39.9	.789	.218	4.470	.532	1.54	4.22	.0368	.310	.347
34.6	13.026	12.4	30.6	43.0	.802	.245	5.106	.441	1.46	5.04	.0396	.292	.332
35.8	14.406	12.0	29.5	41.5	.836	.240	4.766	.458	1.36	4.70	.0533	.312	.365
38.5	13.071	12.5	30.5	43.0	.748	.274	4.900	.398	1.52	5.06	.0424	.296	.338
44.5	14.006	12.0	29.9	41.9	.730	.200	4.860	.610	1.52	4.90	.0594	.326	.376
50.1	13.520	12.2	29.8	42.0	.853	.179	4.888	.486	1.78	4.46	.0394	.314	.353
53.6	14.649	11.5	29.4	40.9	.812	.220	4.630	.388	1.63	4.74	.0448	.306	.351
55.0	13.229	12.1	28.7	40.8	.876	.274	4.864	.395	1.18	4.66	.0307	.316	.347
57.6	15.942	12.2	31.3	43.5	.773	.298	5.132	.381	1.74	4.98	.0468	.328	.375
57.8	10.403	12.3	30.5	42.8	.722	.218	4.970	.712	1.78	4.76	.0612	.322	.383
59.2	11.994	12.1	28.7	40.8	.650	.232	4.989	.484	1.10	4.30	.0349	.314	.349
E1†	.101	.06	.05	.09	.010	.013	.052	.067	.035	.029	.0008	.0015	.0018
E2†	.83%	.49%	.19%	.21%	1.34%	5.30%	1.06%	12.77%	2.41%	.61%	2.10%	.48%	.53%

†See explanation in text, page 16.

TABLE 5.—*Results of Chemical Analysis*
August Sample

Per Cent Fruit Buds Formed	Fresh Weight grams per 100 Spurs	Per Cent Fresh Weight											
		Soluble Solids	Insoluble Solids	Total Solids	Reducing Sugars	Sucrose	Phlorhizin	Water Soluble Acid Hydrolyzable	Starch	Acid Hydrolyzable	Soluble Nitrogen	Insoluble Nitrogen	Total Nitrogen
2.7	9.332	13.2	35.4	48.6	.622	.370	5.630	.353	2.17	4.76	.0295	.336	.366
8.3	11.660	12.4	35.1	47.5	.673	.302	5.468	.352	2.20	5.28	.0314	.329	.360
13.7	8.692	13.0	36.1	49.1	.678	.298	5.686	.361	1.47	4.76	.0380	.377	.415
14.2	10.694	11.6	34.2	45.8	.568	.208	4.962	.319	2.15	4.50	.0378	.331	.369
15.7	11.888	12.0	33.4	45.4	.698	.150	5.570	.388	1.98	4.94	.0294	.320	.349
16.4	11.406	11.7	33.5	45.2	.598	.242	5.166	.269	2.16	4.62	.0363	.330	.366
18.4	11.524	11.9	33.8	45.7	.635	.312	4.927	.321	2.02	5.08	.0412	.324	.365
18.5	9.061	13.5	37.3	50.8	.732	.330	6.377	.348	2.16	5.58	.0339	.378	.412
19.1	10.072	12.6	35.1	47.7	.614	.289	5.478	.405	2.34	4.80	.0235	.318	.342
20.8	10.600	13.1	38.0	51.1	.719	.351	5.258	.458	2.24	5.69	.0416	.368	.410
22.3	13.034	11.2	33.3	44.5	.650	.162	4.904	.306	1.64	5.06	.0396	.330	.370
25.2	12.285	11.6	33.2	44.8	.589	.208	4.462	.276	2.19	4.87	.0386	.336	.375
28.7	9.630	13.0	35.0	48.0	.612	.246	5.568	.353	1.98	4.98	.0462	.401	.447
29.4	11.666	11.7	33.1	44.8	.615	.213	5.006	.276	1.52	4.12	.0628	.383	.446
33.3	12.130	12.6	36.1	48.7	.571	.311	5.314	.402	1.98	5.64	.0354	.355	.390
34.5	13.042	11.1	33.4	43.5	.602	.234	4.678	.300	1.32	4.92	.0510	.338	.389
34.6	11.484	12.3	35.4	47.7	.606	.260	5.354	.380	1.93	4.94	.0374	.343	.380
35.8	12.590	11.5	33.1	44.6	.646	.164	5.168	.296	1.89	4.38	.0490	.374	.423
38.5	13.800	11.8	34.0	45.8	.568	.215	4.888	.294	2.19	4.78	.0440	.343	.387
44.5	14.274	12.1	34.6	46.7	.727	.224	5.011	.333	1.96	5.04	.0582	.370	.428
50.1	12.136	12.2	33.2	45.4	.629	.185	5.256	.356	1.92	4.64	.0514	.357	.408
53.6	14.518	11.7	33.7	45.4	.650	.220	4.654	.292	2.04	4.42	.0568	.356	.413
55.0	13.812	12.6	35.2	47.8	.715	.348	5.365	.434	2.38	5.26	.0339	.342	.376
57.6	10.492	13.4	35.6	49.0	.654	.280	5.726	.308	1.82	5.44	.0475	.404	.452
57.8	9.820	12.4	34.7	47.1	.548	.282	4.966	.300	2.11	5.34	.0535	.398	.452
59.2	14.820	11.7	35.5	47.2	.612	.252	4.836	.346	1.90	5.24	.0382	.354	.392
E1†	.065	.03	.07	.08	.010	.012	.088	.014	.032	.040	.0008	.0024	.0027
E2†	.56%	.25%	.21%	.17%	1.64%	4.53%	1.69%	4.07%	1.61%	.81%	1.84%	.69%	.69%

†See explanation in text, page 16.

various plots when only four observations are used for the calculation of the error. For this reason a standard deviation has been calculated which is based upon the deviations of all the separate samples *from the mean of their own plot.* From this the probable error of the mean of any one plot has been calculated. This error is given, as E_1, at the foot of each column in Tables 4 and 5, and in order to make more evident the relative accuracy with which each substance was determined this error is also stated, as E_2, in terms of its percentage of the mean value for all the plots. It was thought unnecessary to calculate these errors for both bases, since the percentage error would be the same in either case.

The accuracy of most of the determinations is fairly satisfactory. The rather large errors of the sucrose determinations are due to the fact that the actual amounts are rather small, and the errors in reducing sugars are included in the estimation. Uniform washing of the very dry samples used in the determination of water soluble acid hydrolyzable material is hard to effect. This may account for the fairly high error of this determination in the August sample. No explanation can be given for the very high error in the July sample. It would seem that this error should influence the results for starch, but while the starch error is greater in the July than in the August sample, it is not extreme in either case.

INTERPRETATION OF THE RESULTS

A brief inspection of the data shows that little can be learned by comparing individual plots, at least until some general principles have been established. Potter and Phillips (1927) have pointed out that by selecting individual cases from these data support can be found for almost any conclusion. A comparison of Plots 8.3 and 34.5 (July sample, per cent fresh weight) indicates that an increase in phlorhizin tends to be associated with a decrease in the formation of fruit buds. A comparison of plots 14.2 and 28.7 indicates the reverse.

There can be no doubt that the formation of flower buds is the resultant of many influences. It is probable that Plot 28.7 produced more fruit buds than Plot 14.2, not because of its greater phlorhizin content but because of some other circumstances especially favorable to fruit bud formation, and it may well be, in spite of increased phlorhizin. It is not possible to study the phlorhizin content of the spurs of a series of plots in which the nitrogen, starch and all other constituents are constant. It is necessary to reach the best conclusions possible from the analysis of samples in which all the substances determined vary at the same time.

An excellent aid in classifying the relationships involved is the plotting of the amounts of a constituent against the per cent of fruit buds formed. Figure 2 is the graph obtained in this way when phlorhizin (July sample per cent fresh weight) is used. Even though other substances are affecting the results, if phlorhizin is a factor a general tendency should be evident for the dots representing phlorhizin to be either higher or lower as fruit bud formation increases. A line fitted to these dots by the method of least squares shows that on these particular plots, in the season of 1925, there

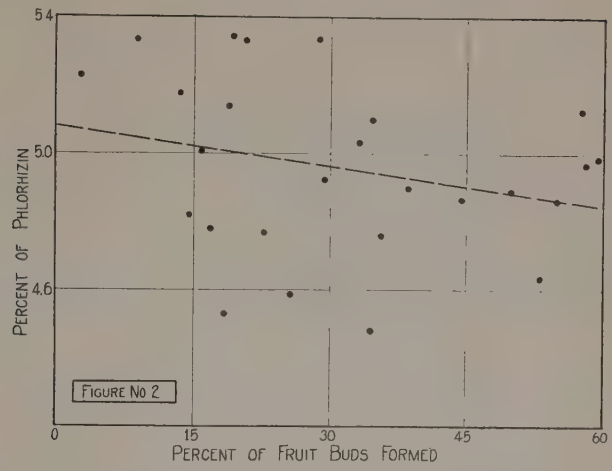

Figure 2. Relation of fruit bud formation to phlorhizin in per cent fresh weight, July sample. Correlation coefficient, —.257 ±.124

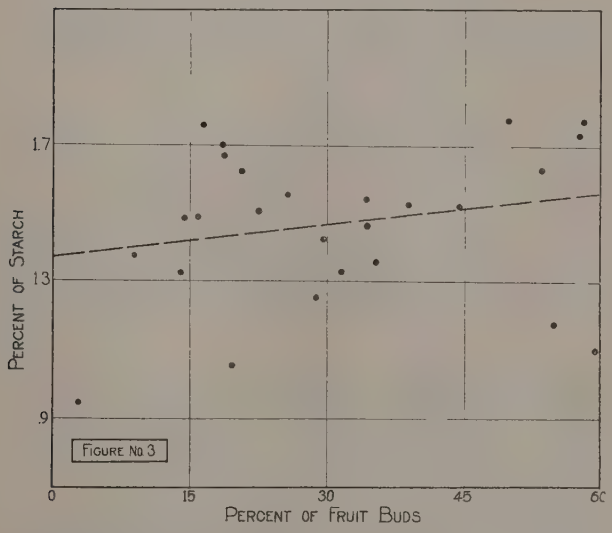

Figure 3. Relation of fruit bud formation to starch in per cent fresh weight, July sample. Correlation coefficient .220 ±.126

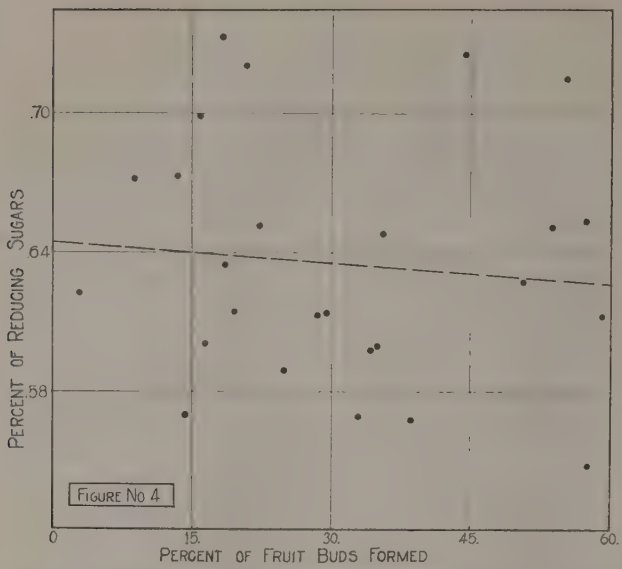

Figure 4. Relation of fruit bud formation to reducing sugars in per cent fresh weight. August sample. Correlation coefficient, —.089 ±.131

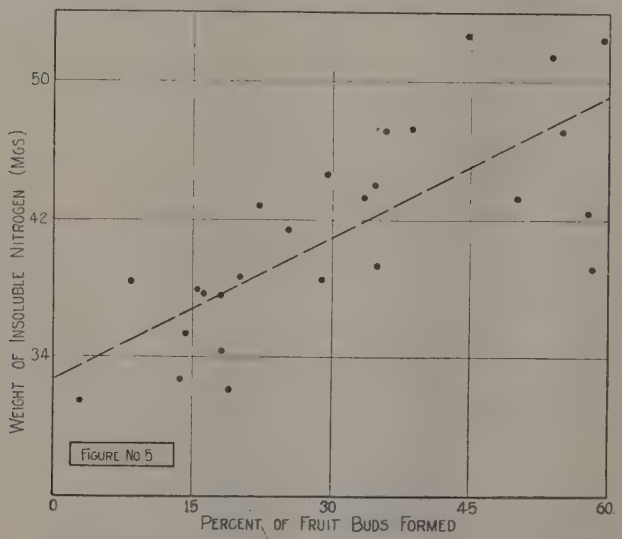

Figure 5. Relation of fruit bud formation to insoluble nitrogen in milligrams per 100 spurs. August sample. Correlation coefficient .750 ±.058

was a tendency for increased fruit bud formation to be associated with a decrease in the per cent of phlorhizin in the spur.

The extent to which the relation between phlorhizin and fruit bud formation is influenced by other conditions is shown by the tendency of the points on the graph to scatter. If this relationship were direct, and not influenced by other constituents, the values for phlorhizin plotted against fruit bud formation would form a line which would be straight or curved depending on the nature of the relation. In that event any other constituent plotted against fruit bud formation would show a complete scatter indicating no relation to high or low fruit bud formation. (See Figure 4).

The values of all the constituents determined, and for both sampling periods, have been plotted in this way against the per cent of fruit buds formed. Space allows the presentation of only a few of these graphs (Figures 2, 3, 4, and 5).

Inspection of these charts shows fairly well whether relationships exist, and, in a general way, how close the relationship is. It is quite evident, however, that an exact method of stating the varying degrees of relationship is desirable. If throughout the range observed the trend in values of the two variables is uniform, that is, if the points tend toward a straight line rather than a curve, the correlation coefficient may be used as an accurate measure of the extent to which changes in one variable are associated with proportional changes in the other. Since none of the graphs constructed showed a tendency toward curvature, this method was adopted. The relation between two variables is expressed by a coefficient varying from 1.0 which shows perfect proportionality, to zero which indicates no relation at all.

The correlation coefficient is a measure of the uniformity with which changes in one variable tend to be associated with changes in the other. It does not measure the size of the increments by which one variable changes in response to unit changes in the other. It expresses in mathematical terms the degree to which any given relation is consistent for all the data, and thus indicates the extent to which other factors are of greater or less importance than the one under consideration. A positive coefficient shows that both variables tend to increase or decrease together, and a negative coefficient indicates that one variable tends to decrease as the other increases.

In calculating correlation coefficients the method of Wallace and Snedecor (1925) has been followed. Their formulæ, which are derived from the more usual forms, are well suited to use with a small number of observations because they adapt the operations of calculation to standard machines so that the work may be done easily and quickly, without introducing the error due to grouping of the data. The complete list of coefficients calculated in this way is given in Table 6.

In this study a considerable amount of data was gathered in order to avoid mistaking relations which are merely coincidental for those which are

TABLE 6.—*Direct Correlations of Substances Determined to Per Cent Fruit Buds Formed*

Substance	July Sample			August Sample		
	Wt. per 100 Spurs	Per Cent Fresh Wt.	Per Cent Dry Wt.	Wt. per 100 Spurs	Per Cent Fresh Wt.	Per Cent Dry Wt.
Fresh Wt.	**.540**	—	—	**.530**	—	—
Sol. Solids	**.502**	−.109	−.311	**.614**	−.107	−.074
Insol. Solids	**.547**	.088	.305	**.583**	−.086	.076
Total Solids	**.518**	.158	—	**.572**	−.104	—
Reducing Sugars	.361	−.242	−.111	.370	−.089	−.075
Sucrose	.145	−.294	−.247	.219	−.158	−.138
Phlorhizin	.450	−.257	−.336	**.460**	−.305	−.421
Water Sol. acid Hydrolyzable	.038	−.190	−.194	.358	−.104	−.087
Starch	**.453**	.220	.212	.358	−.083	−.064
Acid Hydrolyzable	**.471**	−.023	−.014	.449	.114	.200
Sol. Nitrogen	**.488**	.399	.334	**.632**	**.559**	**.526**
Insol. Nitrogen	**.607**	**.456**	.438	**.750**	**.459**	**.546**
Total Nitrogen	**.606**	**.458**	.431	**.744**	**.532**	**.562**

TABLE 6A.—*Levels of Significance Corresponding to Different Values of the Correlation Coefficient*

Value of r	Odds for its Significance
.330	9 to 1
.388	19 to 1
.453	49 to 1
.496	99 to 1

really significant. While it is several times better to depend on 26 observations than on two or four, the number is by no means sufficient entirely to eliminate the possibility of error due to coincidence. If in drawing numbers in pairs, as from a bag, it were found that the numbers of each pair tend to be similar, a correlation would be established based on coincidence alone. The likelihood of any correlation being due to this cause is usually estimated by means of its probable error. If a coefficient is 3.2 times its probable error, one may determine from suitable tables that the odds are 30 to 1 that it is significant rather than accidental. Such odds will not frequently be obtained unless an actual relation exists, but higher odds indicate still greater reliability. The odds are a function of the size of the coefficient and the number of observations on which it is based. Hence, for any group of coefficients based on the same number of observations, one may give directly the odds corresponding to different values of the coefficients. In this paper this seems to be the most simple and adequate way of expressing the relative reliability of the many coefficients given. Fisher (1928) has suggested, however, that the odds may be more accurately determined by the use of a statistic which he calls "t" than by the use of the probable error. Since Fisher's method gives lower odds, and is therefore more conservative, it has been used in this paper. In all the tables of correlation coefficients, all values with a significance of

TABLE 7.—*Correlations of Gains, July to August, in Substances Determined, to Per Cent Fruit Buds Formed**

Substance	Wt. per 100 Spurs	Per Cent Fresh Wt.	Per Cent Dry Wt.
Fresh Wt.	.035	—	—
Sol. Solids	.065	.100	.212
Insol. Solids	.094	−.180	−.205
Total Solids	.087	−.297	—
Reducing Sugars	−.076	.159	.091
Sucrose	.062	.049	.072
Phlorhizin	−.037	−.209	−.160
Water Sol. Acid Hydrolyzable	.139	.146	.165
Starch	−.041	−.198	−.193
Acid Hydrolyzable	.013	.119	.166
Sol. Nitrogen	.032	.023	.064
Insol. Nitrogen	.194	.305	.330
Total Nitrogen	.167	.350	.350

*For levels of significance see Table 6A.

49-1 or higher odds are printed in bold faced type.

A striking feature of Table 6 is the uniformly positive and generally high relation to fruit bud formation of all constituents on the basis of weight per 100 spurs. The values for fresh weight, total solids, and insoluble solids, all of which may be considered as measures of the size of the spur, are uniformly high. It seems definitely indicated that large spurs have a greater tendency to form fruit buds than small ones. Since the large spur tends to contain large amounts of most of its constituents these tend on this basis to show a positive correlation to fruit bud formation. It should be noted, however, that the values for the weight of insoluble nitrogen per 100 spurs are still higher than those for the size of the spur. The question as to which is the really significant relation will be discussed in a later paragraph.

The results on the percentage bases present quite a different picture. Nitrogen is the only substance showing a relation to fruit bud formation which may be considered established beyond question. Reducing sugars, sucrose, phlorhizin and water soluble acid hydrolyzable substances show uniformly rather small negative correlations. If any significance attaches to these results it is not because of the size of the coefficients, but because of the consistent negative trend of similar substances. The relations of starch are small and not consistent.

If certain metabolic conditions within the spur favor fruit bud formation it would seem possible that they might also favor the accumulation or depletion of some of the constituents of the spur as the season advances. Thus it was thought possible that conditions favoring the accumulation of starch might also favor the formation of fruit buds. Accordingly, for each substance the gain or loss of each of the 26 plots during the period from July to August has been computed. These have been plotted against fruit bud formation as in the case of the original amounts of each substance. With these diagrams it was found again that there were no curved regression lines and that therefore the correlation coefficient may be used to express them. The values for these relations are shown in Table 7.

TABLE 8.—*Ratios of Carbohydrates to Nitrogen*

WEIGHT PER 100 SPURS	July Sample		August Sample	
	Mean	Standard Deviation	Mean	Standard Deviation
Reducing Sugars to Soluble Nitrogen	22.10	6.61	15.97	4.20
Sucrose to Soluble Nitrogen	7.09	2.49	6.58	2.60
Phlorhizin to Soluble Nitrogen	139.04	39.81	133.77	36.94
Water Soluble Acid Hydrolyzable to Soluble Nitrogen	14.84	5.98	8.71	2.96
Starch to Soluble Nitrogen	40.68	11.95	50.85	16.49
Acid Hydrolyzable to Soluble Nitrogen	129.93	37.76	124.03	32.20
Reducing Sugars to Insoluble Nitrogen	2.57	.23	1.80	.20
Sucrose to Insoluble Nitrogen	.82	.14	.73	.18
Phlorhizin to Insoluble Nitrogen	16.19	.90	14.96	1.26
Water Soluble Acid Hydrolyzable to Insoluble Nitrogen	1.71	.45	.97	.16
Starch to Insoluble Nitrogen	4.79	.79	5.66	.92
Acid Hydrolyzable to Insoluble Nitrogen	15.13	1.22	13.92	1.50
PER CENT FRESH WEIGHT				
Reducing Sugars to Soluble Nitrogen	21.90	6.71	16.08	4.16
Sucrose to Soluble Nitrogen	7.12	2.47	6.59	2.62
Phlorhizin to Soluble Nitrogen	138.88	39.84	132.86	37.62
Water Soluble Acid Hydrolyzable to Soluble Nitrogen	14.81	5.97	8.72	2.97
Starch to Soluble Nitrogen	40.60	11.91	50.92	16.44
Acid Hydrolyzable to Soluble Nitrogen	130.50	37.28	125.88	32.33
Reducing Sugars to Insoluble Nitrogen	2.54	.23	1.81	.18
Sucrose to Insoluble Nitrogen	.83	.14	.73	.18
Phlorhizin to Insoluble Nitrogen	16.18	.91	14.81	1.34
Water Soluble Acid Hydrolyzable to Insoluble Nitrogen	1.71	.45	.96	.16
Starch to Insoluble Nitrogen	4.79	.78	5.66	.92
Acid Hydrolyzable to Insoluble Nitrogen	15.21	1.17	14.10	1.33

It was surprising to find that in no instance was a change in any given material significantly related to the blossom buds differentiated during or just subsequent to this period. The fruit bud formation in those plots in which starch was accumulating was actually slightly lower than in the others, the difference, however, is not significant. The only relation which approaches significance at all is that of the gain in percentage of insoluble nitrogen. Even in this case the odds are only about 9 to 1 that the relation found is not merely a coincidence.

A great deal of interest has been expressed in recent years in the relation between fruit bud formation and the relative supplies or ratios of carbohydrates and nitrogenous materials. Ratios have been calculated on the basis of total carbohydrates or of total carbohydrates excluding acid hydrolyzable substances to the total nitrogen. On the whole, however there seems to have been a tendency to attach greatest significance to ratios of starch to nitrogen. With the present data, it is possible to calculate a number of different ratios between carbohydrate substances and soluble or insoluble nitrogen. In order to study thoroughly relations of this nature, all possible combinations have been calculated both on the basis of percent fresh weight and on the basis of amounts per 100 spurs.

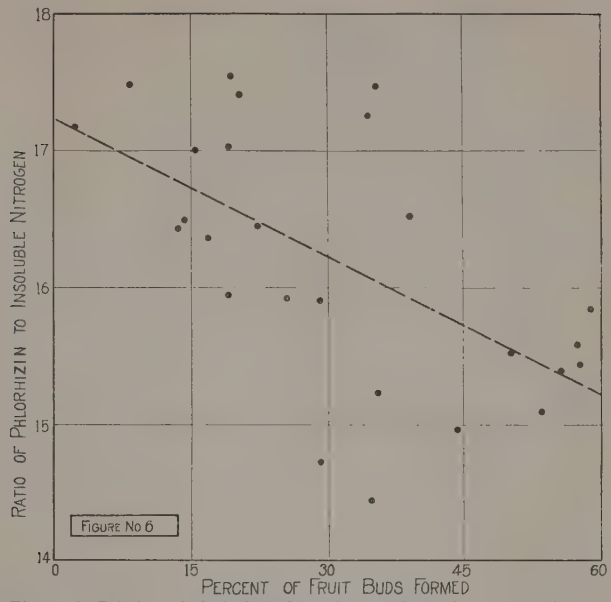

Figure 6. Relation of fruit bud formation to the ratio of phlorhizin to insoluble nitrogen, July sample, per cent fresh weight. Correlation coefficient, —.594 ± .086

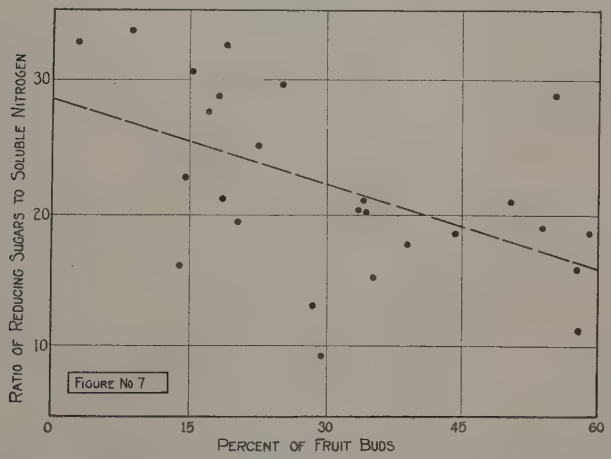

Figure 7. Relation of fruit bud formation to the ratio of reducing sugars to soluble nitrogen, July sample, weight per 100 spurs. Correlation coefficient, —.528 ±.095

Rather than publish approximately 1250 ratios, there are presented in Table 8 the mean value and standard deviation for each series. Since as a rule two-thirds of the individual values will be greater or less than the mean by an amount less than the standard deviation, and only about 4.6 per cent vary by more than twice that amount, this table serves to give a good idea of the range in ratios found in this material.

The ratios, as in the case of the substances themselves and the differences, have been plotted against fruit bud formation. Since on the basis of the well-known hypothesis of Kraus and Kraybill (1918) one would not expect a linear relation, two representative graphs are shown in Figures 6 and 7. These indicate that throughout the entire range observed with these 26 plots of trees, there is a constant negative relation between the carbohydrate-nitrogen ratio and fruit bud formation. Within this range there seems to be no tendency for maximum fruit bud formation to be associated with the medium values of the carbohydrate-nitrogen ratios. Throughout, it appears that the greater the amount of nitrogen and the less the supply of carbohydrate, the more blossoms are formed. The other graphs are similar to those published. Since these relations are linear in nature, it is possible to present them in a concise and accurate way by means of correlation coefficients. These are shown in Table 9. They seem on the whole to indicate that the carbohydrate-nitrogen ratio is as closely related to fruit bud formation in this series of fruit spurs as is nitrogen itself, but no closer.

In interpreting data such as those presented here, the possibility of interrelations existing among the various substances must be considered. One can conceive of a substance, which of itself tends to inhibit fruit bud formation, being essential to the production of another substance which favors the differentiation of flower buds. The coefficients listed in Table 6 represent the net result of the whole situation, in case any such relations exist. This hypothesis can be tested, at least partially, by further statistical analysis.

It has been stated that it is not possible in work of this sort to keep all

TABLE 9.—*Correlations of Fruit Bud Formation to Various Carbohydrate-Nitrogen Ratios**

Carbohydrate Substances	Basis of Wt. per 100 Spurs				Bases of Per Cent Fresh Wt.			
	Ratio to Sol. Nitrogen		Ratio to Insol. Nitrogen		Ratio to Sol. Nitrogen		Ratio to Insol. Nitrogen	
	July	Aug.	July	Aug.	July	Aug.	July	Aug.
Reducing Sugar	−.528	−.556	−.363	−.436	−.537	−.537	−.442	−.390
Sucrose	−.526	−.423	−.384	−.292	−.545	−.422	−.407	−.285
Phlorhizin	−.548	−.570	−.598	−.691	−.548	−.555	−.594	−.620
Water Soluble Acid Hydrolyzable	−.492	−.415	−.268	−.273	−.492	−.418	−.266	−.272
Starch	−.292	−.449	+.062	−.280	−.397	−.451	+.065	−.275
Acid Hydrolyzable	−.488	−.474	−.177	−.275	−.522	−.469	−.294	−.256

*For levels of significance see Table 6A.

TABLE 10.—*Correlations to Fruit Bud Formation in Spurs of Variable and of Constant Size. July Sample*

Substance	Wt. per Spur	
	Spur Size Variable	Spur Size Constant
Reducing sugars	.361	—.274
Sucrose	.145	.025
Phlorhizin	.540	—.051
Starch	.453	.109
Acid Hydrolyzable substances	.471	—.025
Soluble Nitrogen	.488	.182
Insoluble Nitrogen	.607	.329

variables except one constant, in order to study the effect of that one factor alone. However, by means of appropriate formulæ which have been developed by biometricians, substantially the same results may be obtained through the use of the partial correlation coefficient*. It has been shown in Table 6 that fruit bud formation shows a positive relation to size of spur and to all constituents on the basis of weight per spur. It may be desired to know what the relations to various substances would have been had the samples been taken only from spurs of uniform size. From the original data the relation of fresh weight to fruit bud formation has been calculated (.540) and also that of the weight of reducing sugars to fruit bud formation (.361). The data make possible the calculation of a third relation, that of the total weight of reducing sugar to the fresh weight of the spur (.875). From these, by the method of partial correlation it is found that the relation of the total amount of reducing sugar to fruit buds in spurs of uniform size, would be —.274. This value represents the relation existing in these plots when that part of the variability in amount of sugar which is due to variations in size of spur is eliminated from consideration. That the result is numerically the same as would be obtained by the laborious method of collecting samples of spurs of closely similar weight is made clear by examples in both of the references cited.

The relations for total amounts of seven important spur constituents on the bases of variable and of constant size of spurs are shown in Table 10.

Partial correlation tests the hypothesis previously suggested by eliminating from consideration any secondary effects of the variables studied which take place through the variable eliminated. For instance, if large size of the spur is associated with high fruit bud formation merely because in large spurs there is more nitrogen present, a partial correlation of fruit bud formation to fresh weight *with nitrogen constant* will no longer show a positive relation. Such is actually the case, the value being —.169. When size of spurs is no longer a criterion of the supplies of nitrogen (and sub-

*For an excellent discussion of the significance and use of the partial correlation coefficient the reader is referred to Fisher, "Statistical Methods for Research Workers." Revised Edition pp. 152-8. London 1928. and Pearl, "Medical Biometry and Statistics," Chapter 15. Philadelphia 1923.

stances normally associated with nitrogen) it is not associated with the production of bloom.

It is evident that the more completely the complicating factors due to variations in constituents can be eliminated the more nearly the resulting figure will represent the real relationship between size of spur and fruit bud formation. The same considerations apply to each of the constituents. With each constituent that is considered constant, however, a larger coefficient is required for a given level of significance. In Table 6A it is shown that, with 26 observations, a coefficient of .388 has 19 chances of being significant to one of being coincidental. If six variables are held constant this same order of significance requires a coefficient of .444. In this case the calculation, for instance, of the partial correlation coefficient of fresh weight to fruit bud formation with all the substances determined held constant would result in a figure which would have no practical meaning. In order to make use of this valuable method of interpretation it was necessary, then, to select a smaller number of the variables for study by this method. Those chosen were fruit bud formation, fresh weight, reducing sugars, sucrose, phlorhizin, starch, acid hydrolyzable material, soluble nitrogen, and insoluble nitrogen. Soluble, insoluble and total solids and total nitrogen were omitted because they are not sufficiently specific, and water soluble acid hydrolyzable substances because the determination was not very exact, and because of lack of knowledge as to the nature of the material determined. The calculations were made by the method described by Wallace and Snedecor (1925).

The partial correlation coefficients obtained for fresh weight to fruit bud formation were, for the July sample .012 and for the August sample —.083, both of which are entirely without significance. It can be assumed, then, that for these samples fresh weight of spurs in itself is not a significant factor in fruit bud formation and it can be omitted from further consideration. The partial correlation coefficients calculated from the variables mentioned except fresh weight are listed in Table 11, and in Table 11A are given the levels of significance applying to the coefficients in Table 11.

It is possible to state the significance of these partial correlation coefficients in a somewhat clearer way by using the value which Fisher (1928) calls "z" to calculate the range within which the true value of the partial correlation coefficient may reasonably be expected to lie. The extent of this range depends on the size of the estimated partial correlation coefficient and the number of samples used. The ranges determined in this way are presented in Table 12.

This table shows very clearly which of the relationships may be considered as established for the kind of spurs used in this study. The positive relation between insoluble nitrogen and fruit bud formation is emphasized again. It has persisted in all previous methods of stating the results, and whether or not other constituents are held constant. In the July sample, the odds are high for the significance of the negative relation of reducing sugars to fruit bud formation. In all previous methods of statement this coefficient has been negative except on the basis of weight per spur, Table

TABLE 11.—*Partial and Multiple Correlation Coefficients of Substances Determined to Per Cent Fruit Buds Formed*

Substance	July Sample			August Sample		
	Wt. per 100 Spurs	Per Cent Fresh Wt.	Per Cent Dry Wt.	Wt. per 100 Spurs	Per Cent Fresh Wt.	Per Cent Dry Wt.
Reducing Sugars	**−.617**	−.373	−.506	−.288	−.040	.101
Sucrose	−.470	−.272	−.373	.294	−.181	−.038
Phlorhizin	−.426	−.483	−.431	−.228	−.153	−.344
Starch	−.194	−.081	−.231	.029	.243	.277
Acid Hydrolyzable	.404	.348	.343	−.306	.303	.317
Soluble Nitrogen	−.266	−.111	−.120	−.284	.220	−.089
Insoluble Nitrogen	**.692**	**.543**	**.609**	**.677**	.158	.442
Multiples to all Substances	**.828**	**.731**	**.735**	**.866**	**.704**	**.735**

TABLE 11A.—*Levels of Significance Corresponding to Different Values of the Correlation Coefficient*

Value of r	Odds for its Significance
.378	9 to 1
.444	19 to 1
.516	49 to 1
.561	99 to 1

TABLE 12.—*Accuracy of Estimates of Values of Partial Correlations to Fruit Bud Formation (Six Variables Constant)*

Substance	July Sample		August Sample	
	Estimated Partial r	Range Within Which True Value of Partial r Will Fall*	Estimated Partial r	Range Within Which True Value of Partial r Will Fall*
Reducing Sugars	−.617	−.845 to −.201	−.288	−.671 to +.217
Sucrose	−.470	−.773 to +.006	+.294	+.675 to −.210
Phlorhizin	−.426	−.749 to +.061	−.228	−.634 to +.277
Starch	−.194	−.613 to +.310	+.029	+.497 to −.452
Acid Hydrolyzable Material	+.404	+.738 to −.089	−.306	−.682 to +.198
Soluble Nitrogen	−.266	−.658 to +.239	−.284	−.669 to +.221
Insoluble Nitrogen	+.692	+.878 to +.323	+.677	+.872 to +.298

*Odds are 30 to 1 that true value is within the indicated range.

6, where it has a positive value. The method of partial correlation has shown that this positive value is due to the relation of reducing sugars to other constituents of the spur, and is replaced by a negative one when these are held constant. Although some other relations, such as those of sucrose, phlorhizin, and acid hydrolyzable material in the July sample seem probable, they cannot be considered as established beyond question.

The results for the August sample differ very materially from those for the July sample. All the coefficients are considerably lower, except those

TABLE 13.—*Mean Values of Constituents, July and August*

Weight per 100 Spurs	July	August	Gain
Fresh Weight g.	12.14 ± .20	11.71 ± .22	—.43 ± .30
Soluble Solids g.	1.48 ± .02	1.42 ± .02	—.06 ± .03
Insoluble Solids g.	3.57 ± .07	4.00 ± .08	.43 ± .11
Total Solids g.	5.05 ± .10	5.42 ± .09	.37 ± .13
Reducing Sugar mg.	95.90 ± 2.18	74.00 ± 1.71	—21.90 ± 2.77
Sucrose mg.	30.46 ± .80	29.52 ± .86	—.94 ± 1.17
Phlorhizin mg.	601.58 ±10.05	612.92 ± 8.94	11.34 ± 13.5
Water Soluble Acid Hydrolyzable mg.	62.70 ± 1.84	39.59 ± .98	—23.11 ± 2.08
Starch mg.	179.00 ± 5.24	232.31 ± 5.76	53.31 ± 7.79
Acid Hydrolyzable mg.	564.58 ±12.37	573.92 ±13.00	9.34 ±17.94
Soluble Nitrogen mg.	4.811 ± .248	4.949 ± .201	.138 ± .319
Insoluble Nitrogen mg.	37.331 ± .733	41.281 ± .783	3.950 ± 1.073
Total Nitrogen mg.	42.142 ± .935	46.230 ± .961	4.088 ± 1.341
Per Cent of Fresh Weight	**July**	**August**	**Gain**
Soluble Solids	12.21 ± .05	12.23 ± .09	.02 ± .10
Insoluble Solids	29.57 ± .12	34.65 ± .17	5.08 ± .21
Total Solids	41.78 ± .20	46.88 ± .26	5.10 ± .33
Reducing Sugar	.779 ± .008	.636 ± .007	—.143 ± .011
Sucrose	.253 ± .006	.256 ± .008	.003 ± .010
Phlorhizin	4.960 ± .033	5.218 ± .054	.258 ± .063
Water Soluble Acid Hydrolyzable	.523 ± .017	.339 ± .006	—.184 ± .018
Starch	1.465 ± .029	1.987 ± .034	.522 ± .045
Acid Hydrolyzable	4.655 ± .034	4.965 ± .052	.310 ± .062
Soluble Nitrogen	.0391 ± .0017	.0418 ± .0013	.0027 ± .0021
Insoluble Nitrogen	.3069 ± .0019	.3536 ± .0033	.0467 ± .0038
Total Nitrogen	.3460 ± .0033	.3954 ± .0042	.0494 ± .0053

showing the relation of nitrogen to fruit bud formation. That decided changes in composition have occurred during this 30-day period is shown by the listing, in Table 13, of the mean values for each constituent at the two sampling periods and also the changes that occurred.

The fresh weight of the spurs decreased slightly, but the dry weights increased about 0.37 g. per 100 spurs. The net increase in the substances determined was about one-tenth of this amount. It is assumed that the remainder of the increase is due to such substances as cellulose and lignin. The amount of water in the spurs decreased by between 8 and 9 percent of the amount present in the July sample. It is reasonable to suppose that the free water decreased still more, since acid hydrolyzable material (including pentosans) and protein both increased. There is also a shift in the relations among the carbohydrates, shown by decreases in reducing sugars and water soluble acid hydrolyzable material, a slight increase in acid hydrolyzable material and a marked increase in starch. These changes agree with those found by Spoehr (1919) in cacti. Similar changes have been observed accompanying decrease in moisture during the ripening of seeds.

In view of this shift in equilibrium among the groups of carbohydrates it is not surprising that the relations of these substances to fruit bud formation at the two periods should be strikingly different. Since the differentiation of fruit buds was undoubtedly well begun at the later period the metabolic changes due to this process may have had an appreciable effect on the amount and character of the reserves. In that case the composition

TABLE 14.—*Method of Estimating Fruit Bud Formation From Spur Constituents*

Substance	Mean Wt. per 100 Spurs All Plots	Wt. per 100 Spurs Plot 20.8	Deviations Plot 20.8 From Mean	Regression Coefficients Fruit Bud Formation on Each Substance Per Cent per mg.	Tendency of Deviations in Each Substance to Raise or Depress Fruit Bud Formation
	mg.	mg.	mg.		Per Cent
Reducing Sugar	95.9	86.1	9.8	−1.095	+10.7
Sucrose	30.5	24.6	5.9	1.273	+ 7.5
Phlorhizin	601.6	585.0	−16.6	− .189	+ 3.1
Starch	179.0	178.0	− 1.0	− .077	+ 0.1
Acid Hydrolyzable Substances	564.6	524.0	−40.6	.138	5.6
Soluble Nitrogen	4.81	4.44	0.37	· 2.397	+ 0.9
Insoluble Nitrogen	37.3	33.7	3.6	6.792	−24.5
Sum all Substances					− 7.8

Mean of fruit bud formation =31.1%
Estimated fruit bud formation = 31.1 — 7.8 or 23.3%

of the spurs in August may be related to fruit bud formation because of the effect of that process on composition. From the statements made in the section on "Time of Sampling" it would seem reasonable to suppose that relations of composition in July to fruit bud formation may be due to the effect of composition on fruit bud formation.

If the partial correlation coefficients listed in Table 11 really represent the chemical condition of the spurs in relation to fruit bud formation and if the constituents studied are those whose relations to fruit bud formation are most important, then it should be possible, with some degree of accuracy to reverse the process, that is, to estimate from their composition the percentage of spurs forming blossoms with the help of the relationships that have been established. Thus the coefficients show that, other things being equal, the more reducing sugars a spur contains the less likely it is to form blossoms, and the more insoluble nitrogen it contains the greater the probability that it will form a fruit bud, and so on for each of the constituents determined. The actual percentage of blossoms in a given sample should be obtained as the net result of these various and sometimes conflicting influences. The calculation of "regression coefficients" makes such an estimate possible. The size of the regression coefficient for each substance depends on the range in amount of that substance in the samples taken and on the correlations among the variables used in determining the partial correlation coefficients. In the cases to be presented, the regression coefficient is the numerical increase or decrease in percent of fruit buds

TABLE 15.—*Fruit Bud Formation Estimated from Absolute Amounts per Spur of Reducing Sugars, Sucrose, Phlorhizin, Starch, Acid Hydrolyzable Substances, Soluble and Insoluble Nitrogen*

Observed Per Cent	Estimated Per Cent Basis July Sample	Estimated Per Cent Basis August Sample
2.7	7.7	12.9
8.3	14.9	18.3
13.7	32.6	17.3
14.2	19.3	19.3
15.7	9.7	7.6
16.4	31.5	22.2
18.4	20.3	17.3
18.5	19.6	18.9
19.1	16.2	10.8
20.8	23.1	24.9
22.3	16.9	22.7
25.2	38.5	30.0
28.7	35.3	34.6
29.4	30.8	37.1
33.3	22.7	39.4
34.5	37.2	31.1
34.6	23.2	25.4
35.8	41.4	47.6
38.5	35.2	46.9
44.5	53.6	47.2
50.1	41.5	30.5
53.6	48.3	53.3
55.0	35.0	42.6
57.6	56.4	44.6
57.8	40.7	44.3
59.2	55.8	62.2

formed to be expected because of one miligram increase in the substance in question. These coefficients were calculated by the method described by Wallace and Snedecor (1925). The use of the coefficients in estimating fruit bud formation in Plot 20.8, using the composition on the basis of weight per 100 spurs at the July sampling, is illustrated in Table 14. In this case the result, 23.3%, is 2.5% higher than the actual fruit bud formation as determined by counting spurs.

This method, or rather a somewhat shorter one which is the same in principle, has been applied to all the plots using the data from both sampling dates. These estimates, together with the observed values are given in Table 15.

The estimated values, of course, are far from being identical with the observed ones, yet a second random determination of fruit bud formation would deviate considerably from the first. The agreement seems excellent when it is considered that only seven factors are used in making the estimates. The closeness of agreement is shown clearly by the coefficients of correlation between the estimated and observed values. For the July sample this is .828 and for the August sample .866, which show a very close relationship. On referring to Table 11 it will be seen that these coefficients are the same as the multiples listed in the Weight Per 100 Spurs columns. In fact they have the same meaning in both places. They indicate the total extent to which fruit bud formation is determined by the amounts of the constituents listed, or by the same causes that determine the amounts of these constituents. They also show how closely values for fruit bud formation, estimated from the amounts of the constituents, will agree with the observed values. From the multiple correlation coefficients listed in Table 11 it is evident that in estimating fruit bud formation more reliable results are obtained by expressing the composition of the spurs on the basis of weight of constituent per 100 spurs than by expressing it in terms of per cent.

It was thought that a study of the interrelations among the substances determined might show something as to the conditions favoring the accumulation of insoluble nitrogen, which is of such evident importance in fruit bud formation. It was thought possible, too, that other relations of physiological interest might appear. Accordingly, the seven variables previously listed, omitting fruit bud formation, were used in calculating all the possible partial relations. The results are stated in Tables 16 and 17 and the corresponding levels of significance are given in Table 17A.

While these figures are of interest, most of them are small and, since they were determined from only 26 observations, their significance is not great. The positive relation between soluble and insoluble nitrogen is clearly established.

As a further aid in interpreting the results obtained, attention is called to environmental factors which may have caused variations in blossom formation and in the composition of the spurs. The conditions of culture varied from unfertilized sod to cultivation with large applications of nitrogen. So far as growth is concerned the nitrogen supply is without

TABLE 16.—*Partial Correlation Coefficients*
July Sample

	Wt. per 100 Spurs	Per Cent Fresh Wt.	Per Cent Dry Wt.
Red. Sug.-Sucrose	−.373	−.119	−.278
Red. Sug.-Phlorhizin	−.068	−.074	−.210
Red. Sug.-Starch	−.190	−.141	−.254
Red. Sug.-Acid Hydrolyzable	**.513**	.249	.355
Red. Sug.-Soluble Nitrogen	−.211	−.101	−.185
Red. Sug.-Insoluble Nitrogen	.422	.089	.386
Sucrose-Phlorhizin	.235	.145	−.031
Sucrose-Starch	−.397	−.428	−.380
Sucrose-Acid Hydrolyzable	.390	.130	.114
Sucrose-Soluble Nitrogen	−.077	.089	−.172
Sucrose-Insoluble Nitrogen	.115	−.080	.196
Phlor.-Starch	−.095	−.336	**−.633**
Phlor.-Acid Hydrolyzable	.289	.441	−.138
Phlor.-Soluble Nitrogen	−.168	**−.157**	−.139
Phlor.-Insoluble Nitrogen	**.597**	.367	.093
Starch-Acid Hydrolyzable	.473	.424	.084
Starch-Soluble Nitrogen	.013	.295	.010
Starch-Insoluble Nitrogen	.214	−.153	.039
A. H.-Soluble Nitrogen	−.163	−.028	−.066
A. H.-Insoluble Nitrogen	.016	−.171	−.382
Sol. N.-Insoluble Nitrogen	**.660**	**.761**	**.594**

TABLE 17.—*Partial Correlation Coefficients*
August Sample

	Wt. Per 100 Spurs	Per Cent Fresh Wt.	Per Cent Dry Wt.
Red. Sug.-Sucrose	−.020	−.072	−.046
Red. Sug.-Phlorhizin	**.516**	**.618**	**.536**
Red. Sug.-Starch	.273	.072	−.031
Red. Sug.-Acid Hydrolyzable	.387	.412	.207
Red. Sug.-Soluble Nitrogen	.353	.492	**.542**
Red. Sug.-Insoluble Nitrogen	−.246	−.470	**−.515**
Sucrose-Phlorhizin	−.110	.190	−.182
Sucrose-Starch	.216	.239	−.003
Sucrose-Acid Hydrolyzable	.299	.377	−.008
Sucrose-Soluble Nitrogen	−.102	−.002	−.279
Sucrose-Insoluble Nitrogen	−.003	−.003	.125
Phlor.-Starch	.012	−.154	−.085
Phlor.-Acid Hydrolyzable	−.003	−.445	−.352
Phlor.-Soluble Nitrogen	−.361	**−.802**	**−.749**
Phlor.-Insoluble Nitrogen	.479	**.778**	**.608**
Starch-Acid Hydrolyzable	.013	−.026	−.087
Starch-Soluble Nitrogen	−.289	−.249	−.062
Starch-Insoluble Nitrogen	.240	.060	−.166
A. H.-Soluble Nitrogen	−.441	**−.546**	−.309
A. H.-Insoluble Nitrogen	**.526**	**.561**	−.178
Sol. N.-Insoluble Nitrogen	**.881**	**.882**	**.865**

TABLE 17A.—*Levels of Significance Corresponding to Different Values of the Correlation Coefficient*

Value of r	Odds for its Significance
.369	9 to 1
.433	19 to 1
.503	49 to 1
.549	99 to 1

doubt the most important factor. In Table 18 the several plots are grouped according to the equivalent quantity of nitrogen received. Fruit bud formation and the percentages of some of the more important fruit spur constituents are included. For this purpose, since it is known that cultivation releases nitrogen from the soil, it has been assumed that cultivation furnishes as much nitrogen to the tree as an application of 5 lbs. of nitrate of soda per year. It may be seen that the amount of nitrogen available has not affected significantly the proportion of fruit buds formed. The amount of soluble nitrogen is significantly higher in the 10-12 than in the 0-2½ series, although the relatively large probable error indicates that individual plots vary widely. Probably the treatment has increased insoluble nitrogen also, although the data are not conclusive. That such differences should be found is to be expected on the basis of evidence previously contributed by Hooker (1922, 1924) Kraybill et al. (1925) Shrader and Auchter (1925) Lagasse (1926) and Thomas (1927).

It is known that the production of a crop of apples tends to inhibit blossom formation, either because of the initial nutritional drain of blossoming or because of the demands of the developing fruit. Unfortunately, a record of fruit production was not obtained on all of these plots. The record of the average yield of apples per tree for 18 of the plots and the fruit bud formation observed are shown in Table 19. In order to bring out certain phases of the relations which appear to exist, the percentage of fruit bud formation has been plotted against the weight of fruit borne by the trees (Fig. 8). Taking into consideration the whole 18 plots, there appears to be no definite relation between the amount of crop borne and fruit bud formation, the correlation coefficient is .070 which is entirely without significance. An examination of the data indicates that there are five plots, 2.7, 18.4, 18.5, 19.1, and 20.8, which bore little fruit and yet for some other reasons not definitely known failed to form many fruit buds. The correlation coefficient between weight of crop and per cent of spurs forming flower primordia for the remaining 13 plots is —.351, which indicates a tendency for blossom production to decline as the crop increases. Considering the number of observations this correlation coefficient could by no means be taken as indisputable evidence that fruiting decreases the formation of flower buds. However, since it is well known that this is ordinarily the case, it seems reasonable to conclude that, in the present instance, some of the plots failed to form fruit buds because there were many blossoming spurs and developing fruits on the trees and that other

TABLE 18.—*Effect of Culture on Composition and Performance of Baldwin Spurs*

Equivalent Sodium Nitrate Lbs. per Tree	Number of Plots	Per Cent Spurs Forming Fruit Buds	Fresh Wt. 100 Spurs July	Typical Constituents, July, in Weight per 100 Spurs						
				Reducing Sugar	Sucrose	Phlorhizin	Starch	Acid Hydro-lyzable Substances	Sol. Nitrogen	Insol. Nitrogen
0— 2½	5	28.9	11.612	93.4	32.7	593.	141.	550.	3.62	35.0
4— 5	7	29.3	12.408	94.3	30.1	619.	176.	563.	4.71	38.2
7— 8	7	29.7	12.007	91.0	29.5	584.	201.	560.	4.40	36.3
10—12	6	35.3	12,275	99.3	29.2	603.	185.	568.	6.28	39.2
Error of Difference Between Two Series		6.5	.607	6.7	2.5	31.	16.	38.	.76	2.3

TABLE 19.—*Relation of Size of Crop to Fruit Bud Formation*

Plot	Av. Wt. Fruit per Tree, lbs.	Plot	Av. Wt. Fruit per Tree lbs.
19.1	11	55.0	162
2.7	14	25.2	183
20.8	48	15.7	209
18.4	58	8.3	241
59.2	76	14.2	252
18.5	81	53.6	290
44.5	119	34.5	355
38.5	130	13.7	390
16.4	149	29.4	451

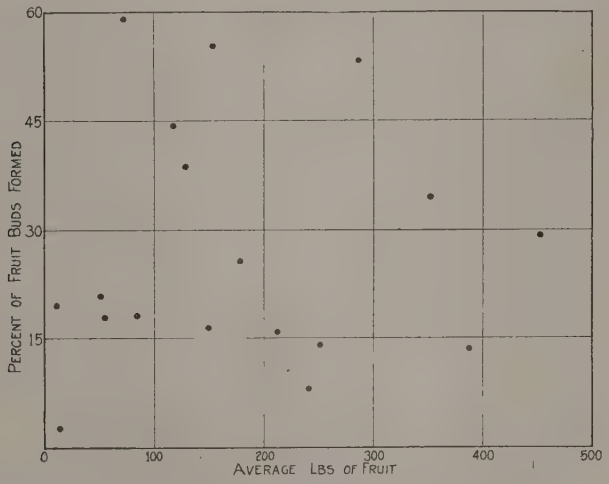

Figure 8. The relation of fruit bud formation to the average weight per tree of the 1925 crop.

plots failed to form fruit buds for some other unknown reason.

Any relations which can be established between composition and fruit bud formation which will hold for trees in which blossom formation was inhibited because of fruiting and also for those in which it was inhibited by some unknown condition would seem to be capable of fairly general application to orchard conditions.

DISCUSSION

The accuracy with which the correlation coefficient measures any physiological relation depends upon (1) the importance of the relation as compared with others affecting the same measurable characters and (2) the

number of observations. Thus if perfect correlation exists between two variables a few observations measure it as accurately as 1000. In that case there is but one variable with two criteria of measurement. Similarly, high correlations may be determined with comparatively few observations. On the other hand, if, for instance, one factor has but slight effect on fruit bud formation, its influence would be masked almost constantly by that of other more important factors, and a very large number of observations would be needed to determine whether it had any influence at all.

After correlations are measured there remains the problem of interpreting their meaning. If one of two correlated phenomena could not possibly have influenced the second, it is fair to assume that the second either caused the first or that the two are due to a common cause. The height of daughters, for instance, can scarcely be a factor affecting height of fathers, and any correlation must be due to what the daughter inherits from her father or to what both inherit for a common ancestry. However, as Fisher (1928) points out, this correlation may be due in part to a tendency for tall men to choose or be chosen by tall wives, and on this basis a partial correlation of height of fathers to daughters with height of mothers constant would be lower than the direct correlation.

It is believed that for the season of 1925, in which the observations were made, the correlations presented in the preceding section have shown that a complex relation existed between fruit bud formation and the constituents of the spur, in which the amount of insoluble nitrogen present was by far the most important single component. The nitrogen relation is sufficiently close to be established beyond reasonable doubt, and even measured with some degree of accuracy under the conditions of this experiment. It does not seem likely that the processes which give rise to flower embryos, the first microscopic evidence of which is visible about August first, can have affected appreciably the composition of the spurs in early July. Nor does it seem probable that mere accumulation of insoluble nitrogenous material can affect the course of development of the meristem. The storage of nitrogen, therefore, would seem to be due to the same causes as the formation of fruit buds. It is an indication of those conditions within the spur which favor the reproductive function. The data at hand afford little aid in defining these conditions aside from the close relation between soluble nitrogen and insoluble nitrogen shown in Tables 16 and 17.

The evidence indicates that there are conditions other than those favoring accumulation of insoluble nitrogen which, taken in the aggregate, have a considerable bearing on fruit bud formation. These cannot be defined individually, but the uniformly negative trend of the partial correlation coefficients of the soluble carbohydrates (July sample) to fruit bud formation indicates that these may be concerned.

These conclusions, although based on fairly consistent results and a relatively large amount of data, do not agree well with opinions now in vogue concerning fruit bud formation. They indicate clearly that bearing and non-bearing spurs cannot be placed in the same category, since bearing spurs have been found rather consistently to have the higher nitrogen con-

tent, but they always produce less bloom[1]. On Baldwin trees they very rarely produce any bloom.

The possibility must be considered that in the bearing spur the nitrogen content is higher than the optimum. Three sets of analyses in which only new growth was included in the sample offer the best evidence available on this question. Shrader and Auchter (1925) in a series of five plots, found the total nitrogen content on June 27, on the dry weight basis, to range from 0.777 to 0.939 per cent in non-fruiting spurs and from 1.034 to 1.460 in fruiting. Harley (1925) gives composition data for one plot in two seasons. In 1923, the earliest analysis given, August 8, shows the rather unusual result of 1.61% total nitrogen, on the dry weight basis in non-bearing, and 1.50% in bearing spurs. The following year this situation was reversed and the percentages were 2.24 and 2.30, respectively. Lagasse (1926) found in three plots that the total nitrogen, per cent dry weight, varied from 0.653 to 1.145 in non-blossoming spurs according to treatment. Corresponding fruiting spurs varied from 1.297 to 2.071%.

These analyses, although made with different varieties and in various localities, seem rather consistent. They also agree well with the present work in which, on the dry weight basis, the maximum nitrogen content of the non-bearing spurs was 1.023% (Plot 29.4). They indicate quite definitely that the range in nitrogen content is higher for fruiting than for non-fruiting spurs, but also that the two ranges may be expected to overlap somewhat under varied cultural conditions. That in the short span between the two the optimum nitrogen content is passed, and the transition to almost complete inhibition from over-supply of nitrogen is reached seems unlikely. Finally if evidence from spurs including some "old growth" may be permitted, in two sets of comparable bearing spurs Kraybill et al. (1925) found the only tendency to bloom displayed by those of the higher nitrogen content. In no other case has the tendency to bloom of bearing spurs of different nitrogen content been determined. At present the safest conclusion is that bearing and non-bearing spurs are not directly comparable. In fact, this conclusion might be inferred from the differences in amount and character of tissues concerned.

The results of this study were surprising since they failed to show that the accumulation of carbohydrates, especially starch, favors fruit bud formation. Yet the case for starch, so far as direct evidence from analysis is concerned, depends almost entirely on comparisons of bearing and non-bearing spurs. Other evidence is meagre and conflicting. Kraybill et al. (1925) found in two sets of non-bearing spurs that those with less starch were more fruitful. It was concluded that the spurs from the sod plot were too high in carbohydrates, including starch. Yet the difference between the two types of spurs in this case may be explained adequately without ascribing any significance to starch, on the basis of the relations used in this paper for estimating fruit bud formation from composition. Hooker's data (1920) indicate that non-bearing fruit bud forming spurs of

[1]Exceptions to this statement are known in certain cases with McIntosh, but they are rare.

Jonathan and Ben Davis were higher in starch June 26 than permanently barren spurs of one tree each of Ben Davis and Nixonite. While no counts of blossom bud production were made it is quite certain that reproduction was greater in the spurs having high starch content. Nor can their behavior be explained on the basis of other constituents, such as nitrogen or reducing sugars, at least on a percentage basis. Absolute amounts per spur were not calculated.

The results of Lagasse (1926) show material differences in starch accumulation in non-bearing spurs from different fertilizer plots. No counts were made, but it seems certain that if any differences existed most blossoms were formed on the plots fertilized with nitrogen. Spurs from these plots are relatively high in nitrogen and low in starch. Starch accumulation alone will not account for this performance in any case, and if the fertilized plots produced more bloom the results fit well with those reported here.

Potter, et al. (1930) have studied deflorated spurs which in a sense are non-bearing. If all the spurs on a heavily blooming tree were deflorated considerable growth took place in the secondary shoots; fruit buds were formed and starch accumulated somewhat. If only half the spurs were deflorated and the remainder allowed to bloom and set fruit, growth and fruit bud formation were depressed and less starch accumulated. On a percentage basis, the nitrogen content was somewhat lower during June in the spurs from trees 50% deflorated but after early July was about the same in both cases. While the absolute amounts per spur were not determined it is obvious that the spurs from trees completely deflorated had more because of their greater size. This seems to constitute a rather unusual case in which both starch content as percentage or absolute amount and nitrogen on the basis of weight per spur are correlated with greater reproduction.

Using only the data available for non-fruiting spurs from trees under ordinary culture, it is difficult to show that starch content is the determining factor in blossom production. High nitrogen content does not seem to fit every case perfectly, but on the whole seems more consistently correlated to flower formation.

The general belief that blossoming is associated with increase in carbohydrates is supported by the results of various special practices. Defoliation and shading are known to depress blossom formation, and ringing increases it. The effect of these practices on composition has been studied by Kraybill (1923) who found the twigs of shaded Oldenburg apple trees lower in sugar and higher in nitrogen than comparable checks. Roberts (1927) working with young Wealthy trees found the same situation and also less starch. Auchter, et al. (1926) working with Stayman Winesap obtained similar results with all substances except reducing sugars which seemed slightly higher on a percentage basis in the shaded spurs.

Kraybill (1923) Harvey (1923) and Roberts (1927) have studied the effect of ringing on the composition of apple tissues. Although the portions sampled varied from shoots to two year old wood the results are all

similar, showing, on a percentage basis, higher sugars, much higher starch and lower nitrogen. For new shoots there seems to be a corresponding increase in acid hydrolyzable substances in the ringed plants. Curtis (1923) presents rather clear evidence that nitrogen does not move into a ringed branch in appreciable amounts. Hence increased blossoming could be associated with an increase in nitrogen content only on the basis of the rather remote possibility that the nitrogen of the tissues above the ring migrates to the spurs. There is no direct evidence on this point. Harvey and Murneek (1921) found that defoliation, which depressed flower formation in apple spurs, caused an apparent increase in reducing sugars. Proebsting, examining the bark and wood, found at first a decrease and later an increase of monosaccharides in defoliated McIntosh trees. Harvey (1923) obtained almost identical results with defoliated shoots of Spitzenberg and Grimes. As regards sugars the results are not exactly as might be anticipated, indicating only a temporary decrease. Starch, however, is consistently decreased by defoliation and on the percentage basis nitrogen is increased. Since growth is usually checked by defoliation the absolute amounts of nitrogen may be smaller in defoliated trees. In one case Roberts (1923) found a higher percentage of nitrogen at the nodes of defoliated than of normal plum shoots. There can be no reasonable doubt that in these instances fruit bud formation is associated with increased carbohydrate reserves and generally with decreased nitrogen at least on the percentage basis. There is a chance, as may be seen from Table 12, that the failure to find a positive relation between flower formation and carbohydrate in this study is due merely to coincidence. This is unlikely and the evidence from fruit spur composition studies does not warrant the serious consideration of this rather remote possibility. At present it seems preferable to seek some other means of reconciling these results with those of special practices.

Less drastic and more nearly normal practices, such as grafting on slow growing stocks, tend to promote blossom bud formation. It has been natural to ascribe this result, as in the case reported by Swarbrick (1928), to the accumulation of carbohydrates following the check in growth. However, the data of Curtis (1923) on normal stems and shoots indicate that nitrogen moves into tissues after growth ceases. In leaves of peach and lilac it accumulated at least as rapidly as other substances and in leaves of privet it increased markedly in per cent of the dry weight. Increased nitrogen following a growth check may well be the cause of the increased fruit bud formation in such cases.

In these results the carbohydrate-nitrogen ratios have not proved of special significance. It might be thought that all of the spurs sampled were too high in carbohydrates, approaching condition four of Kraus and Kraybill. Variations in nitrogen would then be the most important factor, if not the only one, in changing the ratio. However, variations in carbohydrate content did occur. In July starch varied from .95 to 2.77 mg. per spur, and reducing sugar from .73 to 1.30 mg. as compared with a range in insoluble nitrogen of from .313 to .528 mg. The variations in nitrogen

were not greater than in other substances, but they were more similar, plot for plot, to those of fruit bud formation.

Another difficulty in harmonizing these results with the Kraus and Kraybill theory and the more recent results of Nightingale et al. (1928) lies in the fact that, as shown in Figures 6 and 7, there is no tendency for the medium ratios to be associated with highest blossom bud formation. This may be due to the fact that the whole range in ratios is small and that all of them lie somewhere between condition four, where unfruitfulness prevails because of too low nitrogen supplies in relation to carbohydrates and condition three in which, with a better balance, maximum fruitfulness is found. This view seems in harmony with the observed fruit bud formation which varied from 2.7 to 59.2 per cent.

While the results of this study place the major emphasis on insoluble nitrogen, that a condition of balance is implied somewhat similar to that proposed by Kraus and Kraybill is shown by an inspection of Table 14, which states the method of estimating fruit bud formation from the chemical composition of the spurs. Although the coefficients for carbohydrates are small, yet, in the example given, low insoluble nitrogen is partly balanced by low soluble carbohydrates so that fruit bud formation is not much below the mean. Should both increase they tend to remain in balance and to indicate at least medium fruitfulness. Should they be out of balance because of high carbohydrate and low nitrogen the method would indicate greatly depressed fruit bud formation, just as would be expected on the basis of the Kraus and Kraybill hypothesis. The main difference lies in the fact that, based on the regression equation, a tree that is out of balance because of high insoluble nitrogen and low carbohydrate would be expected to bloom profusely.

As a matter of fact the over-vegetative unfruitful condition to be expected on the Kraus and Kraybill hypothesis is rarely if ever found among mature orchard trees. It has not been obtained in the Cornell orchards by feeding Wealthy trees 50 to 75 pounds of sodium nitrate per year accompanied by severe pruning, nor at the Maine Station by applying very large amounts of nitrogen to a Rhode Island tree*. Certainly such a condition had not been reached in any of the trees included in this experiment. Other studies of these trees show clearly that those receiving the most nitrogen make the greatest annual growth and produce many more spurs than are formed by trees to which nitrogen has not been applied. As shown in Table 18 the total amount of nitrogen per spur is increased only slightly, and their increased yield is due not to a larger proportion of fruit buds but principally to the fact that they carry a large number of spurs.

Within the range of conditions to which the trees used in this study were subjected, the relation of each of the substances determined to fruit bud formation appears to be linear. There is no doubt that the range over which these linear relationships hold is limited and that maximum or min-

*These statements are based on conversations with A. J. Heinicke and Karl Sax.

imum relations to fruit bud formation would appear if any of the constituents were increased or decreased sufficiently.

In the experiments on defoliation and shading it is quite possible that the maximum of the relation of nitrogen to fruit bud formation has been passed. Whether this is the case cannot be decided on the basis of the evidence at hand. A second possibility is that these practices increase the amount of soluble nitrogen present as compared with the amount of insoluble nitrogen. The coefficients in Table 14 show that such a shift would be expected to result in decreased fruit bud formation. It has been shown that the best estimate of fruit bud formation is made when the amounts of the constituents are expressed as weight per spur. Shading and defoliation result in decreased growth and smaller spurs. Hence there is a third possibility; that the slightly increased per cent of nitrogen in such spurs might not indicate a greater actual amount than in the larger spurs not subjected to such treatments.

In the case of ringing a shift from soluble to insoluble nitrogen would be expected. Such a change, as far as it goes, would tend toward increased fruitfulness. It should be noted, too, that according to the regression equation (Table 14) a large increase in soluble carbohydrates would be balanced by a relatively small increase in insoluble nitrogen, and that starch has very little effect.

Further studies, covering additional seasons and including a wider range of conditions, are needed in order to determine the extent to which the relations found in this work apply.

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